

UNCLASSIFIED

AD NUMBER
AD481043
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Apr 1966. Other requests shall be referred to United States Army Biological Labs., Fort Detrick, MD.
AUTHORITY
BODRL D/A ltr, 28 Sep 1971

THIS PAGE IS UNCLASSIFIED

AD

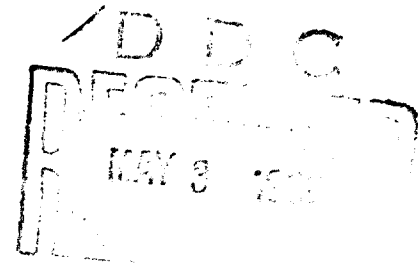
TECHNICAL MANUSCRIPT 259

481043 REPLICATION OF RIFT VALLEY FEVER VIRUS
IN L CELL SUSPENSION CULTURES

Henry R. Tribble, Jr.

John J. Boyle

APRIL 1966



UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

Reproduction of this publication in whole or part is prohibited except with permission of Commanding Officer, U.S. Army Biological Laboratories, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 259

REPLICATION OF RIFT VALLEY FEVER VIRUS
IN L CELL SUSPENSION CULTURES

Henry R. Tribble, Jr.

John J. Boyle

Medical Bacteriology Division
and
Virus and Rickettsia Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1C522301A082

April 1966

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

It has been possible to propagate Rift Valley fever (RVF) virus and obtain high virus yields in L cell suspension cultures maintained in a chemically defined medium. Peak titers ($10^{6.0}$ to $10^{6.5}$ plaque-forming units/ml) were obtained in 24 hours and were comparable to plaque titers obtained in monolayer cultures propagated in a serum-containing medium. The addition of stabilizing compounds (bovine albumin and protamine sulfate) at the time of inoculation resulted in increased in vivo titers but no increase was observed in the plaque titers. The addition of serum to the samples prior to storage enhanced the stability of the virus. Substitution of fructose for glucose in the defined medium reduced the virus yields. However, the addition of proline and serine to the fructose-containing medium restored the virus yields to the level obtained with the glucose medium.

REPLICATION OF RIFT VALLEY FEVER VIRUS IN L CELL SUSPENSION CULTURES

One of the first experiments on Rift Valley fever (RVF) virus replication in tissue culture was conducted by MacKenzie in 1933* with minced chick embryo and Tyrode's solution. More recently, many cell lines and primary monolayers have been used for the propagation of this virus. Although serum was necessary for high virus titers from all these cell lines, extremely low yields of RVF virus have been obtained in the absence of serum. Before this report, no information was available on the replication of RVF virus in suspension cell cultures or cell cultures propagated in a chemically defined medium.

The Van Wyk strain of RVF virus used was a third tissue culture passage product. The L cell suspension cultures were propagated in 30 ml of medium in screw-cap 100-ml serum bottles in a New Brunswick gyrotory shaker at 35 C and 128 rpm. L cells were maintained as stock cultures in Nagle's defined medium.** This medium consists of 13 essential amino acids and glutamine, vitamins, glucose, and inorganic salts.

Suspension cultures (1×10^6 cell/ml) were inoculated with 1 cc of the virus to give the desired viral multiplicity and assayed for determination of initial viral titers. Inoculated L cell cultures were incubated at 35 C in the gyrotory shaker for 1 hour, after which the cells were washed once in fresh medium, resuspended, and samples were removed for virus assay and cell enumeration. The viable cells were determined by the trypan blue method in a hemocytometer. The samples were stored at -60 C with 25% heat-inactivated calf serum as a stabilizer unless stated otherwise. Virus titers were determined by the injection of 10- to 14-gram mice with 10-fold dilutions of the samples and expressed as mouse intraperitoneal LD₅₀ (MIPLD₅₀) after calculation by the Reed-Muench method. Plaque-forming units (pfu) titers were obtained by inoculation of chick primary cell monolayers.

Figure 1 presents the pfu and MIPLD₅₀ titers of RVF virus obtained in L cells in defined medium and in defined medium containing 10% calf serum. The zero-time titer represents the cell-associated virus remaining after the L cells were washed following absorption. The medium containing serum yielded a peak titer of $10^{6.8}$ pfu/ml on day 1, followed by a decrease in titer to $10^{5.8}$ pfu by day 4. The virus in defined medium also had a peak pfu titer of $10^{6.8}$ /ml but the titer decreased more rapidly and by day 3

* MacKenzie, R.D. 1933. The cultivation of the virus of Rift Valley fever. J. Pathol. Bacteriol. 37:75-79.

** Nagle, S.C.; Tribble, H.R.; Anderson, R.E.; Gary, N.D. 1963. A chemically defined medium for the growth of animal cells in suspension. Proc. Soc. Exp. Biol. Med. 112:340-344.

it was less than 10 pfu/ml. The in vivo titers obtained in the serum-containing culture yielded a peak MIPLD₅₀ titer of $10^{7.6}$ /ml; in defined medium, a peak titer of $10^{6.4}$ /ml was obtained. Cell lysis in the defined medium was complete by day 2, but, in the serum-containing culture, complete lysis required 4 days. The virus samples in the above experiments were stored in absence of calf serum.

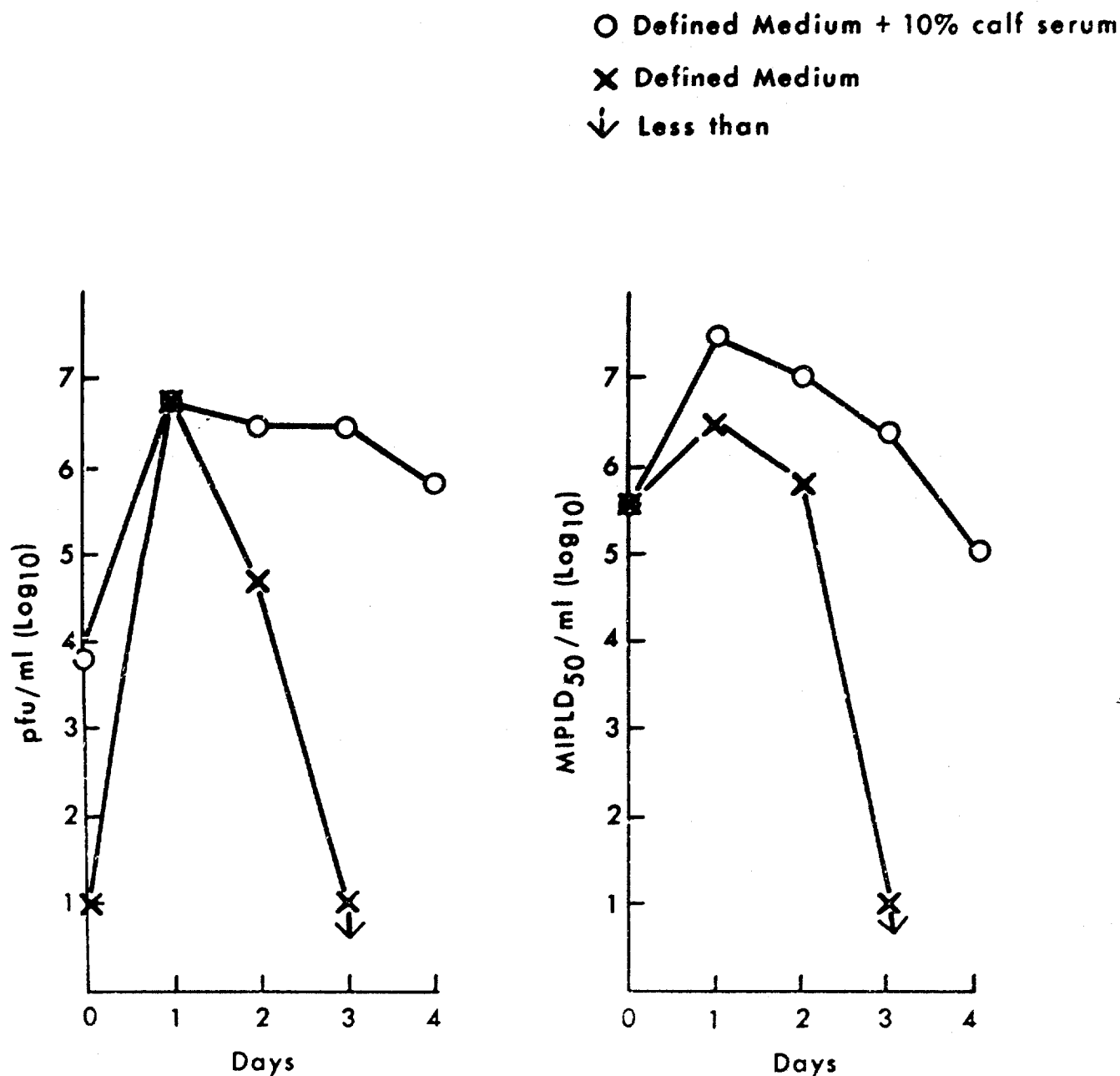


Figure 1. Rift Valley Fever Virus Replication in L Cell Suspension Cultures.

The reduced titers obtained in defined medium may be attributable to a more rapid viral inactivation occurring in a protein-free system rather than to difference in total virus yield. This theory is based on Boyle's report* in 1964 that RVF virus samples in storage maintained higher titers in the presence of serum. To test this possibility, L cells were propagated in defined medium and inoculated with RVF together with either albumin (18 mg/ml) or protamine sulfate (20 μ g/ml) added as stabilizers. The results of these tests are presented in Figure 2. The cultures with added albumin yielded higher peak titers ($10^{7.2}$ pfu and $10^{6.8}$ MIPLD₅₀/ml) compared with titers ($10^{6.8}$ pfu and $10^{6.6}$ MIPLD₅₀) presented in Figure 1 obtained without the stabilizer. The cell lysis was complete by day 2 and the titer declined as rapidly as in the medium without adjuvants. On the other hand, the addition of protamine sulfate at the time of virus inoculation had no appreciable effect on peak viral titers.

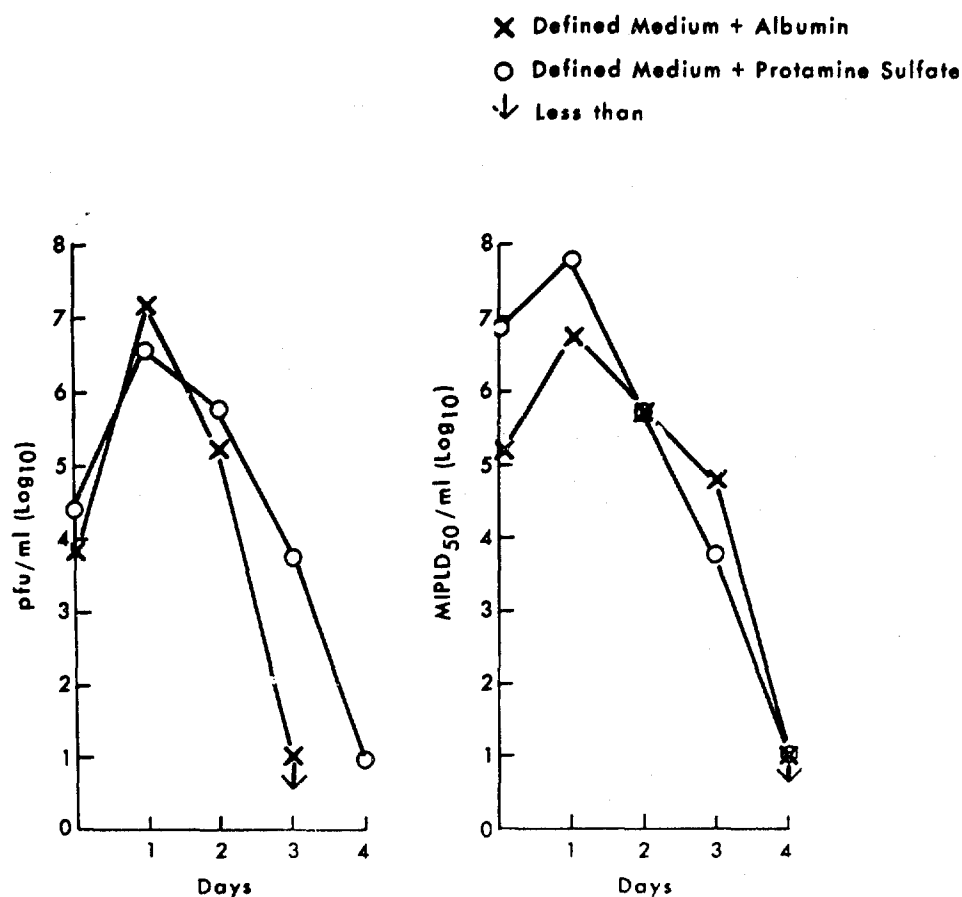


Figure 2. Rift Valley Fever Virus Replication in L Cell Suspension Cultures.

* Boyle, J.J. 1965. Titration of Rift Valley fever in the hamster kidney in the absence of serum. Amer. J. Vet. Res. 26:190-191.

Another factor contributing to rapid cell lysis and affecting the titers obtained in defined medium is virus multiplicity. The virus multiplicity in the previous test had been about 50 pfu per cell. Figure 3 presents data of a test employing varying multiplicities ranging from approximately 1 to 0.001 pfu to each L cell. Albumin (18 mg/ml) was added at the time of viral inoculation and the samples were stored in the absence of serum. Peak titers of $10^{7.4}$ pfu/ml were obtained with multiplicity of 1, 0.1, and 0.01, but a titer of only $10^{6.8}$ pfu/ml was obtained with a multiplicity of 0.001. Complete cell lysis was delayed in all cases until day 4. These data indicate that a multiplicity of one or less is adequate to obtain high viral titers. In view of these results, the effects of protamine sulfate (20 μ g/ml) and albumin (18 mg/ml) added at the time of viral inoculation were again studied. A virus inoculum multiplicity of one was used and all samples were stored in 25% heat-inactivated serum to stabilize the virus. Also, the effect of the addition of proline and serine to the cell culture medium was investigated. These results are shown in Figure 4. The presence or absence of the additives had no effect on peak titers ($10^{8.0}$ pfu/ml). These results, obtained with albumin and protamine sulfate when inactivated serum was present during frozen virus storage, also suggest that the earlier effects of these adjuvants may be attributable primarily to their stabilizing action during storage.

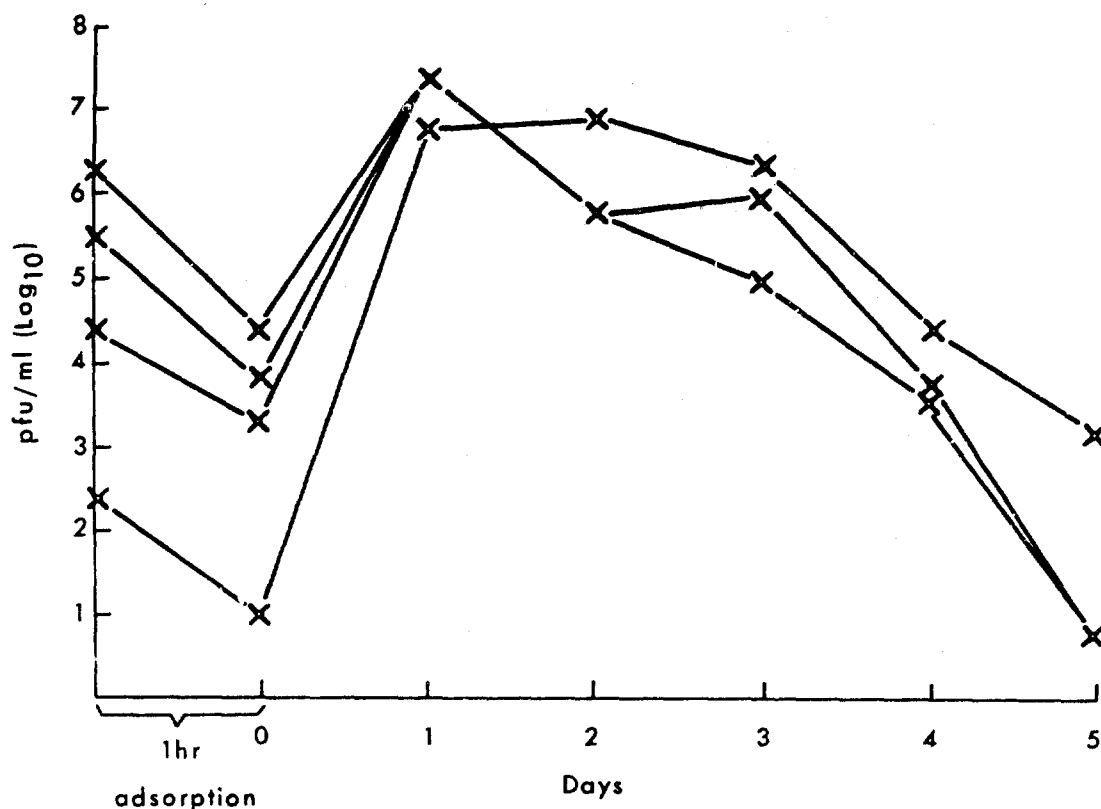


Figure 3. Effects of Virus Multiplicity on Replication of Rift Valley Fever Virus in Defined Medium in L Cell Suspension Cultures.

Another factor contributing to rapid cell lysis and affecting the titers obtained in defined medium is virus multiplicity. The virus multiplicity in the previous test had been about 50 pfu per cell. Figure 3 presents data of a test employing varying multiplicities ranging from approximately 1 to 0.001 pfu to each L cell. Albumin (18 mg/ml) was added at the time of viral inoculation and the samples were stored in the absence of serum. Peak titers of $10^{7.4}$ pfu/ml were obtained with multiplicity of 1, 0.1, and 0.01, but a titer of only $10^{6.8}$ pfu/ml was obtained with a multiplicity of 0.001. Complete cell lysis was delayed in all cases until day 4. These data indicate that a multiplicity of one or less is adequate to obtain high viral titers. In view of these results, the effects of protamine sulfate (20 μ g/ml) and albumin (18 mg/ml) added at the time of viral inoculation were again studied. A virus inoculum multiplicity of one was used and all samples were stored in 25% heat-inactivated serum to stabilize the virus. Also, the effect of the addition of proline and serine to the cell culture medium was investigated. These results are shown in Figure 4. The presence or absence of the additives had no effect on peak titers ($10^{8.0}$ pfu/ml). These results, obtained with albumin and protamine sulfate when inactivated serum was present during frozen virus storage, also suggest that the earlier effects of these adjuvants may be attributable primarily to their stabilizing action during storage.

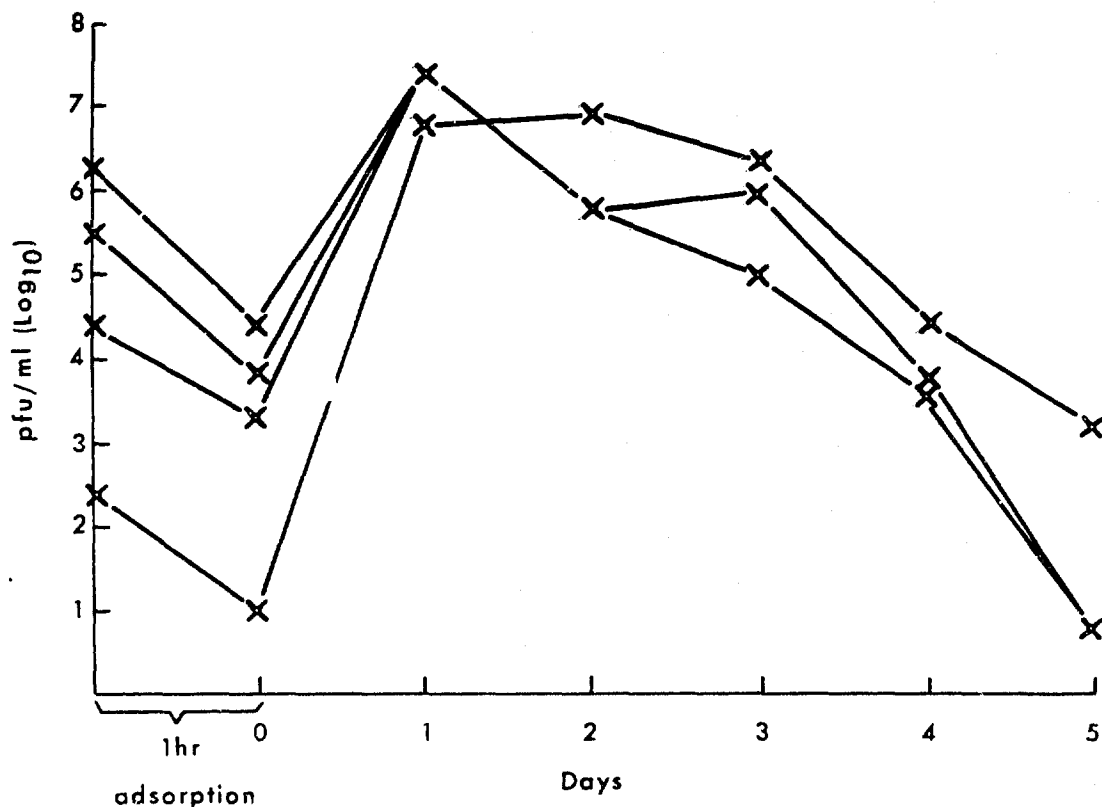


Figure 3. Effects of Virus Multiplicity on Replication of Rift Valley Fever Virus in Defined Medium in L Cell Suspension Cultures.

X 18 mg/ml albumin added
 O 20 µg/ml protamine sulfate added
 Δ No additive

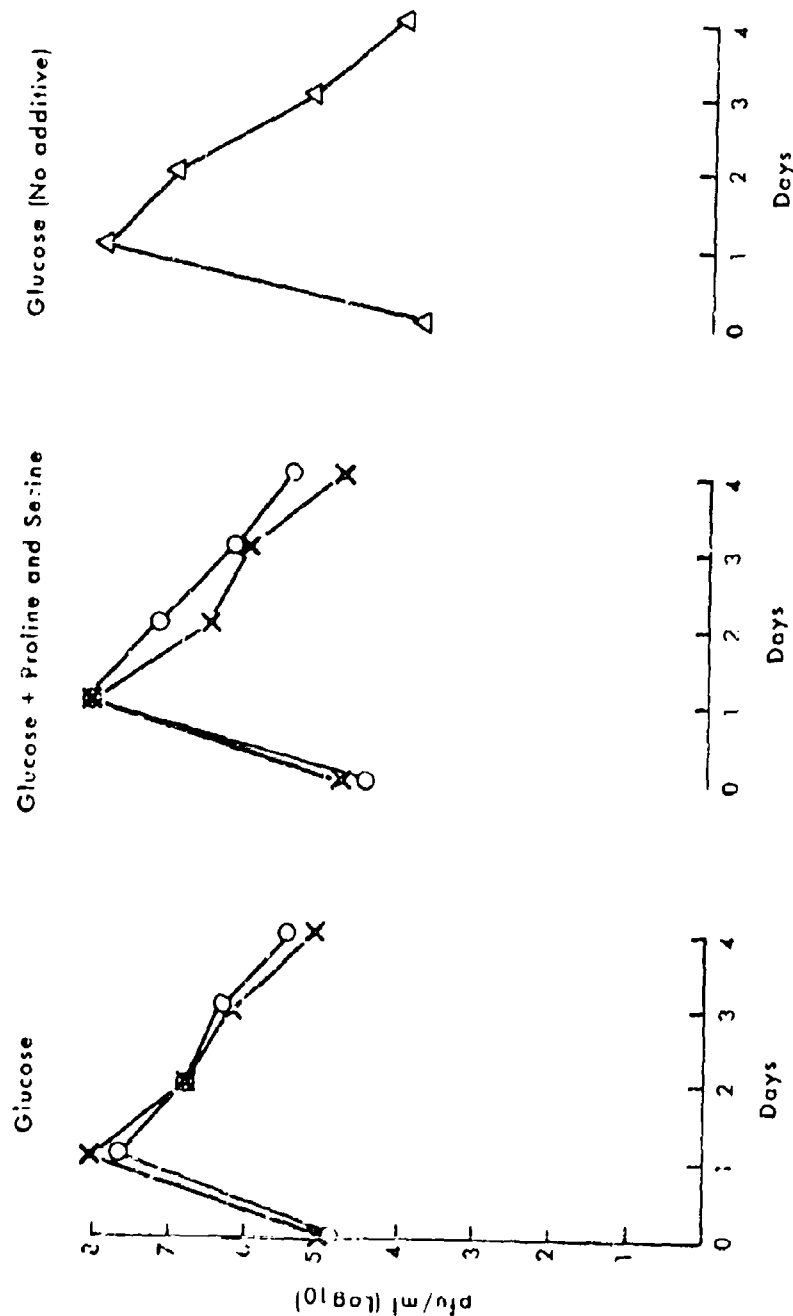


Figure 4. Replication of Rift Valley Fever Virus in L-Cell Suspension Cultures Prepared in Modified Defined Media.

Figure 5 presents results obtained when the defined medium was modified by replacing glucose with fructose. The L cell was adapted to the fructose medium prior to viral inoculation so that cell populations in the fructose and glucose medium were approximately equal. The multiplicity of the viral inoculum was one. The titers obtained in the medium containing fructose were consistently at least $\frac{1}{2}$ a log lower than those obtained in glucose medium.

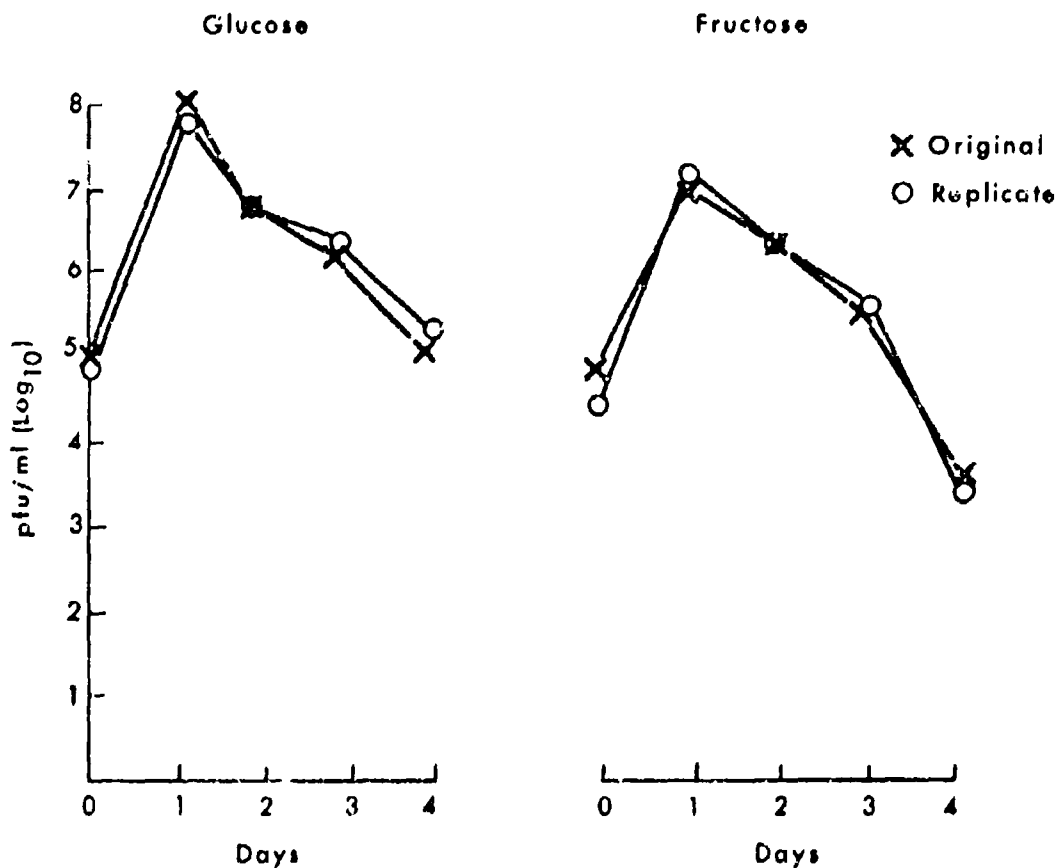


Figure 5. Replication of Rift Valley Fever Virus in L Cells Propagated in Modified Defined Media.

Figure 6 shows results obtained with the addition of proline and serine to the fructose medium. The addition of these amino acids, in contrast to the results obtained in the glucose medium, increased the peak titers to the level obtained in glucose medium.

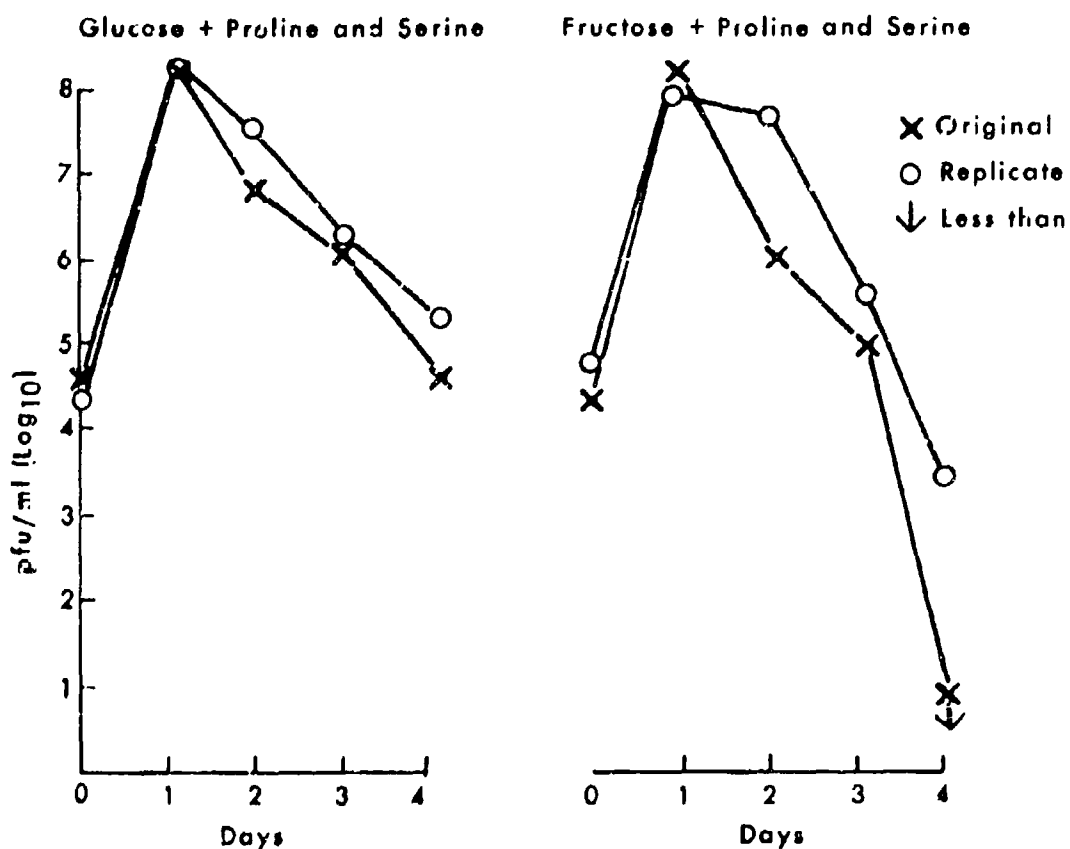


Figure 6. Replication of Rift Valley Fever Virus in L Cells Propagated in Modified Defined Media.

In summary, Rift Valley fever virus will replicate in L cell suspension cultures propagated in chemically defined medium. Titers of approximately 8 logs, as determined by both in vitro and in vivo assay, were obtained in 24 hours. Although high titers were obtained initially with extremely high multiplicities, comparable peak titers could be obtained with multiplicities less than 1. The substitution of fructose for glucose as a carbohydrate source resulted in decreased titers. The addition of proline and serine to glucose medium did not increase the yields; however, the addition of these two amino acids to the fructose medium did increase the peak titer to approximately the level obtained in glucose medium.